JOURNAL OF BACTERIOLOGY, Jan. 1977, p. 1-8
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Vol. 129, No. 1
Printed in U.S.A.

Isolation and Characterization of *Escherichia coli* K-12 F⁻ Mutants Defective in Conjugation with an I-Type Donor

LOUIS HAVEKES,* JAN TOMMASSEN, WIEL HOEKSTRA, AND BEN LUGTENBERG

Department of Molecular Cell Biology and Institute for Molecular Biology, State University of Utrecht, Utrecht, The Netherlands*

Received for publication 23 July 1976

Escherichia coli K-12 F⁻ mutants defective in conjugation with an I-type donor (ConI⁻) were isolated and characterized. These mutants are specific in that they are conjugation proficient with other types of donor strains. They have an altered susceptibility to phages and detergents. Chemical analysis of the cell envelopes of mutant strains has shown that the lipopolysaccharide (LPS) is altered and that one major outer-membrane protein is absent. Conjugation experiments in which LPS from wild-type cells was added to a mating mixture, made up with wild-type donor and recipient cells, showed inhibition in transconjugant formation when an I-type donor, but not an F-type donor, was used. This strongly suggests that LPS of the recipient cell is directly involved in the ability to mate with an I-type donor but not with an F-type donor. The mutations are located in the 78- to 82-min region of the *E. coli* map, with one exception where the mutation maps near or in the galactose operon.

To study the role of the *Escherichia coli* K-12 recipient cell during the early steps in the conjugation process, recipients defective in conjugation may be helpful. The first steps in the conjugation process are attachment of the sex pilus to the surface of the recipient cell and subsequent injection of donor genetic material (for details, see reference 5). It is commonly assumed that the receptor site for the sex pilus consists of part of the outer membrane. Cell envelope alterations in *E. coli* K-12 recipient cells may thus lead to a deficient attachment site for the sex pilus and consequently cause conjugation deficiency.

All the conjugation-deficient (Con⁻) mutants described until now are deficient at least in crosses with F-type donors (ConF⁻). The ConF⁻ mutants that have been tested in crosses with an I-type donor are conjugation proficient (ConI⁺), e.g., strains PC2040, PC2041 (9), and AM3001 (8). The mutant strain F3A (20) is conjugation deficient in crosses with both types of donors. From the genetics and the biochemistry of ConF⁻ mutants (8, 9, 22) it was hypothesized that the heat-modifiable major outer-membrane protein, designated as d, 3a, or II* (for a comparison of the designations used by various groups for the major outer-membrane proteins of *E. coli* K-12, see reference 16a), is the receptor for the F-pilus as well as for phages K3 (22) and TuII* (11). The *tut* or *tolG* gene, the structural gene for this protein, is located near *pyrD* (6, 11).

In this paper we will describe the isolation of *E. coli* K-12 recipient mutants specifically blocked in conjugation with an I-type donor (ConI⁻). Subsequent biochemical characterization shows that these mutants contain an altered lipopolysaccharide (LPS).

MATERIALS AND METHODS

Bacterial strains. All strains are derivatives of *E. coli* K-12. Their relevant characteristics are listed in Table 1. Marker positions, origin, and direction of transfer of the donor strains are given in Fig. 1.

Phages. Laboratory stocks of wild-type coliphages T2, T3, T4, T5, T7, P1, χ (21), K3 (22), Mel (a phage that probably uses outer-membrane protein c [16a] as the receptor [C. Verhoef et al., manuscript in preparation]), and C21 were used.

Media and chemicals. Bacterial cells were cultivated in brain heart broth (brain heart infusion, Difco; 37 g/liter) or peptone-yeast broth (peptone, 1%; NaCl, 0.5%; yeast extract, 0.5%; $Na_2HPO_4 \cdot 2H_2O$, 0.1%), which was previously designated as yeast broth (16a). For plating, peptone-yeast agar or minimal medium (26) agar was used. Required growth factors, amino acids, purines, or pyrimidines were added in final concentrations varying from 20 to 60 μg/ml. If necessary, streptomycin, tetracycline (both from Mycofarm, Delft, The Netherlands), rifampin (a generous gift from Lepetit, Rotterdam, The Netherlands), or mitomycin C (obtained from Kyowa Hakko Kogyo Co., Ltd., Tokyo) was added to a final concentration of 100, 20, 40, or 0.5 μg/ml, respectively. Media were solidified with agar at a final concentration of 1.5%. MacConkey agar was obtained from Oxoid.

TABLE 1. *Bacterial strains*[a]

Strain	Mating type[b]	Sex pili	Relevant characteristics[c]	Source or reference
AB1157	F⁻		*thr leu pro his thi argE lacY galK xyl strA*	Adelberg
AM4000	F⁻		As AB1157 but colicin I resistant	This paper
AM4001 through AM4028	F⁻		As AM4000 but ConI⁻	This paper
D21	F⁻		*trp pro his strA*	(3)
D21e7	F⁻		*lpsA* derivative of D21 with LPS deficient in both galactose as well as in heptose-bound phosphate	(3)
D21f1	F⁻		Derivative of D21e7 that also lacks glucose in its LPS	(3)
CE1007	F⁻		*thr leu strA*, galactose-deficient LPS	(23)
CE1018	F⁻		*thr leu strA*, glucose-deficient LPS	(23)
P6922dI	F⁻		Lacking outer-membrane major proteins b, c, and d	(10)
I53	R144*drd-3*	I	*colI*⁺	E. Meynell
ED102	R64-11	I-like	R_1 Tc Sm *drd*	N. S. Willetts
χ1100	R100-1	F-like	R_F Tc Cm Su Sm Sp *drd*	R. Curtiss III
UB1025	R1*drd-19*	F-like	R_F Km Sm Su Ap Cm	F. Beard
PC0031	HfrR4	F		
PC1511	Hfr KL14	F		
PC0012	Hfr AB313	F	*strA*	
PC0008	HfrH	F		
PC0617	Hfr Cav	F		
PC0515	Hfr KL16	F		

[a] All PC strains were obtained from the Phabagen Collection, State University, Utrecht. All donor strains are streptomycin sensitive unless otherwise indicated.

[b] Marker position, origin, and direction of transfer of donor strains are given in Fig. 1.

[c] The tentative structure of LPS of *E. coli* K-12 is given in Fig. 2.

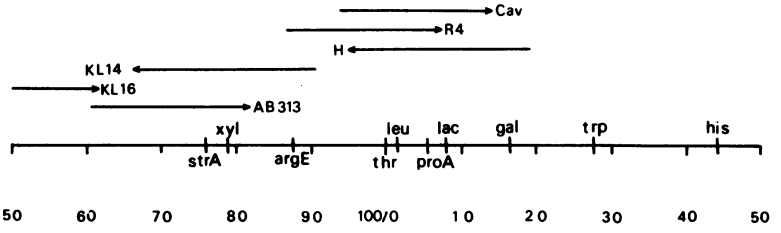

FIG. 1. *Marker position, origin, and direction of transfer of Hfr donor strains. The arrows indicate the position of the origin and the direction of transfer of the Hfr strains used (15).*

Bacterial growth. Growth, represented by an increase in turbidity, was measured in a Klett-Summerson photometer at 660 nm.

Isolation of recipient mutants unable to conjugate with an I-type donor (ConI⁻ mutants). For the isolation of ConI⁻ mutants, we used lethal conjugation as the enrichment procedure. Strain I53, carrying the I-type R factor R144*drd*-3 with genes coding for colicin production, was used as donor, and strain AM4000, pretreated with the mutagenic agent ethyl methane sulfonate, was used as recipient. Brain heart broth was used as the culture medium. Log-phase donor cells, grown to a Klett reading of 30 (2×10^8 to 3×10^8 cells/ml), were mixed with log-phase recipient cells in a ratio of 5 to 1. After 90 min of mating at 37°C, the mating mixture was diluted 100 times in broth supplemented with streptomycin (to kill the donor cells) and mitomycin C. During subsequent aeration at 37°C for 4 h, mitomycin C induces the production of colicin in most of the recipient cells that received the colicinogenic R factor R144*drd-3*. These recipient cells are consequently killed. The mixture was then diluted 100-fold in broth and incubated overnight at 37°C. The overnight culture was used for a second enrichment procedure. After three enrichment cycles, the cell suspension was appropriately diluted and spread on peptone-yeast agar plates containing streptomycin. Colonies that survived the lethal conjugation were picked up and screened for the presence of the donor colicinogenic R factor by testing colicin production by a double-layer test (see below). Surviving colonies unable to produce colicin were tested for recipient ability in a cross with strain

I53(R144*drd-3*) as donor. Survivors that are reduced at least 10-fold in transconjugant formation frequency were isolated and tentatively scored as ConI⁻.

Crosses with the I-type donor I53 carrying the R factor R144*drd-3*. Log-phase donor and recipient cells, grown to a Klett reading of 30 (2×10^8 to 3×10^8 cells/ml), were mixed in a ratio of 1:10 and incubated for 30 min in a water bath at 37°C to allow transfer. Transfer was terminated by violent agitation, using a Low and Wood shaking machine (16). The mating mixture was appropriately diluted and plated on peptone-yeast agar plates containing streptomycin and grown overnight at 37°C. To test the transfer of the colicinogenic R factor, the resulting colonies were killed by exposing them to chloroform vapor for about 20 min. After the chloroform vapor had disappeared, the plates were layered with 5 ml of peptone-yeast soft agar containing about 10^7 cells of a colicin-sensitive indicator strain. After overnight incubation at 37°C, a clear halo was visible around a Col⁺ (colicin-producing) colony.

Crosses with F-type donor strains and donor strains carrying an R factor. Log-phase donor and recipient cells, grown to a Klett reading of 30, were mixed in a ratio of 1:10 and incubated in a water bath at 37°C to allow transfer. Transfer was terminated by violent agitation, and the mating mixture was diluted and spread on selective minimal medium plates (F-donor) or on peptone-yeast plates containing antibiotics (R-donor).

Transduction. Transduction with phage P1 was performed as described by Willetts et al. (25).

Tests for sensitivity to bile salts, sodium dodecyl sulfate (SDS), dyes, and bacteriophages. For these tests we used the methods described earlier (9).

Cell envelope preparation and polyacrylamide gel electrophoresis. For the preparation of cell envelopes and polyacrylamide gel electrophoresis of membrane proteins we used the method described by Lugtenberg et al. (16a).

Isolation of LPS and characterization of the LPS sugar composition. For the isolation and characterization of ^{32}P-labeled LPS by means of paper chromatography, we used the method described by Boman and Monner (3), using isobutyric acid-1 N NH_4OH (5:3, by volume) as the solvent. The LPS used for conjugation inhibition experiments was isolated as described by Galanos et al. (7).

Conjugation inhibition experiments with LPS. LPS was dissolved in distilled water by heating (50°C) and ultrasonic treatment. A 0.1-ml portion was added to 0.1 ml of log-phase donor cells (strain I53, 2×10^8 to 3×10^8 cells/ml). This mixture was then immediately added to 0.9 ml of log-phase recipient cells (strain AM4000). After incubation at 37°C for 30 min, transfer was terminated by violent agitation and the number of transconjugants was determined as described before. A cross with 0.1 ml of water instead of 0.1 ml of LPS solution was used as a control.

RESULTS

Isolation of mutants unable to conjugate with an I-type donor (ConI⁻). Con⁻ mutants, impaired in the first steps of the conjugation process, are cell envelope mutants. It might therefore be possible to isolate ConI⁻ mutants among phage-resistant, antibiotic-resistant, or detergent-sensitive mutants. Such an indirect procedure might result in the isolation of cell envelope mutants in which the ConI⁻ character is merely a pleiotropic effect. We therefore decided to isolate mutants in a more direct way, using lethal conjugation with an I-type donor as an enrichment procedure. In that way we isolated a set of mutants deficient in conjugation with I-type donors. Some of these mutants, all derived from strain AM4000, are described in this paper (strains AM4001, AM4011, AM4012, AM4014, AM4017, AM4018, AM4023, and AM4028).

Sensitivity to bile salts, SDS, and dyes. Cell envelope mutants often have an altered sensitivity to bile salts, detergents, and dyes. All our mutants grew well on MacConkey medium containing bile salts. All mutants except strains AM4014 and AM4018 were unable to grow on peptone-yeast agar with 1% (wt/vol) SDS. All mutants were more sensitive to the dyes acridine orange (0.2 mg/ml) and methylene blue (0.3 mg/ml) than was the parental strain AM4000.

Sensitivity to bacteriophages. Sex pili and bacteriophages may attach to the same or closely related cell envelope components (20, 22). The mutants were therefore tested for a possible altered phage susceptibility. The results are presented in Table 2. The phages T3, T4, T7, and C21 are supposed to use LPS as the receptor (14). The receptor for phage T5 is a protein (4), and that for T2 probably is also a protein (14). Most mutants are, in contrast to the parental strain, sensitive to phage C21, suggesting that their LPS is altered such that it is galactose deficient but contains heptose (24). The C21-sensitive mutants are resistant to phage χ, which uses motile flagella as its receptor (21). An altered LPS could explain this result, since it has been reported that a number of LPS mutants have lost their flagella (1). Strain AM4014, which is still resistant to phage C21 and became resistant to phage χ, might have defective flagella. The resistance of strains AM4023 and AM4012 for phages T2 and T5, respectively, may be the consequence of an indirect effect of changed LPS on the architecture of the outer membrane. It is definitely not the consequence of a double mutation, since SDS-resistant revertants of all SDS-sensitive mutants show a phage pattern identical to that of the parental strain.

Recipient ability. The reduction in recipient ability with an I-type donor (strains I53 and

TABLE 2. *Sensitivity to bacteriophages*[a, b]

Phage	Strain								
	AM4000	AM4001	AM4011	AM4012	AM4014	AM4017	AM4018	AM4023	AM4028
T2	s	s	s	s	s	s	s	*r*	s
T3	s	s	s	s	s	s	s	*r*	s
T4	s	s	s	s	s	s	s	*r*	s
T5	s	s	s	*r*	s	s	s	s	s
T7	s	s	s	s	s	s	s	*r/s*	s
χ	s	*r*	*r*	*r*	*r*	*r*	s	*r*	*r*
C21	r	*s*	*s*	*s*	r	*s*	r	*s*	*s*

[a] r and s stand for resistance and sensitivity, respectively; r/s stands for an efficiency of plating of 0.05.
[b] All strains were sensitive towards phages P1, K3, and Mel.

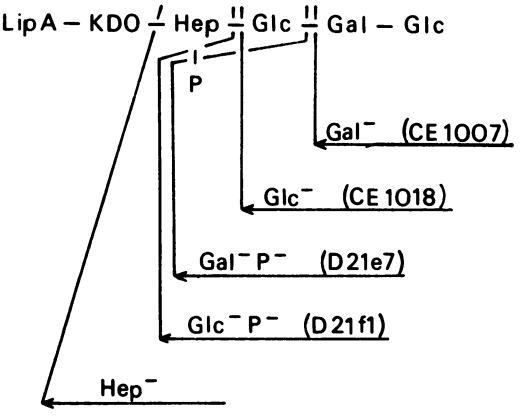

FIG. 2. *Tentative structure of LPS of E. coli K-12. The lipid A-KDO (2-keto-3-deoxyoctonate) region is assumed to be similar to that of E. coli BB (19). One of the heptoses is substituted by phosphate (H. Mayer, personal communication). Sugar analyses of LPS of wild-type E. coli K-12 LPS suggest that KDO, heptose, glucose, and galactose are present in the ratio 3:2:2:1 (B. Lugtenberg and N. van Selm, unpublished data). The order of the sugars was concluded from analysis of various mutants. This structure certainly lacks some constituents that may not be relevant to this paper. In the formula are illustrated the different kinds of LPS of the LPS mutants used in this paper.*

ED102), expressed as the ratio of the number of transconjugants per milliliter in crosses with the parental strain and the number of transconjugants per milliliter in crosses with the mutant strain, varies from 10 to 500 between the different mutants when the cells are cultivated and mated in brain heart broth (Table 3).

The ConI⁻ character of some mutants varied from experiment to experiment. The growth phase of the cells only had a slight influence on the recipient ability in that the reduction factor was slightly lower in stationary-phase cells (results not shown). We also measured the reduction factor of the mutants cultivated and mated in peptone-yeast broth (Table 3). It appeared that strains AM4001, AM4012, AM4017, and AM4028 were approximately wild-type recipients in peptone-yeast broth, whereas the other mutants listed in Table 3 were still poor recipients. Table 3 shows that all mutant strains under all conditions are efficient recipients for F-donor strains. It also appears from Table 3 that the mutants, as far as tested, are good recipients in crosses with donor cells carrying an F-like R-factor. Recipient mutant AM4017 is exceptional in that respect.

Composition of major outer-membrane proteins. The mutants described are ConI⁻ when grown in brain heart broth, but some mutants are ConI⁺ after growth in peptone-yeast broth (Table 3). It has been reported recently that the amount of the peptidoglycan-associated major outer-membrane protein b is much lower after growth in brain heart broth than after growth in peptone-yeast broth (B. Lugtenberg, J. Meijers, R. Peters, P. van den Hoek, and L. van Alphen, FEBS Lett., in press). Protein b could thus be involved in the receptor activity of the ConI receptor. Analysis of the protein patterns of exponential-phase cells grown in brain heart medium showed that protein b was present in the parental strain, strongly decreased in strain AM4014, and absent in all other mutants. In exponential-phase cells of strain AM4000, grown in peptone-yeast broth, the amount of protein b was higher than after growth in brain heart broth. Growth in peptone-yeast broth resulted in wild-type levels of protein b in mutant strain AM4018. All other mutants lacked the protein except strain AM4014, in which reduced levels were present. Comparison of these results with those shown in Table 3 shows that the presence of protein b is not required for ConI proficiency.

LPS sugar composition. Sensitivity to detergents and dyes, the altered phage susceptibility (Table 2), and the changes in the major outer-membrane protein composition could be caused

TABLE 3. *Recipient ability of the ConI⁻ mutants*[a]

Donor type	Sex pili	Medium	Recipient							
			AM4001	AM4011	AM4012	AM4014	AM4017	AM4018	AM4023	AM4028
I53(R144*drd-3*)	I	Brain heart broth	–	–	–	–	–	–	–	–
I53(R144*drd-3*)	I	Peptone-yeast broth	+	–	+	–	+	–	–	+
PC0031(HfrR4)	F	Brain heart broth	+	+	+	+	+	+	+	+
χ1100(R100-1)	F-like	Brain heart broth	+	+	ND[b]	+	–	+	+	+
UB1025(R1*drd-19*)	F-like	Brain heart broth	+	+	ND	+	–	+	+	+
ED102(R64-11)	I-like	Brain heart broth	–	–	ND	–	ND	–	–	–

[a] + and – stand for Con⁺ and Con⁻, respectively. Con⁻ was defined as a more than 10-fold reduction.
[b] ND, Not determined.

by changes in the structure of the LPS (1, 9, 12, 22a). Therefore, LPS from the parental and the mutant strains was labeled with [^{32}P]phosphate, isolated, and compared by paper chromatography, using the technique described by Boman and Monner (3). Figure 3 shows the chromatographic analysis with LPS of the parent, the ConI⁻ mutants, and a number of reference strains. In the solvent used, wild-type LPS (from strains AM4000 and D21), which has the largest polysaccharide moiety, has the lowest R_f value. From the analysis of the reference strains it is clear that an LPS with a shorter polysaccharide moiety has a higher R_f value. The phosphate group bound to heptose decreases the R_f value (compare D21e7 with CE1007 or D21f1 with CE1018). Comparison of R_f values of LPS of the parent and mutant strains (Fig. 3) shows that all mutants contain an altered LPS. The structure of the LPS of the various mutants cannot be identified with certainty, since the "wild-type" strains AM4000 and D21 apparently differ slightly in the structure of their LPS, as can be concluded from the R_f values. When, despite this objection, LPS of references and mutants is compared, the results suggest that the mutant strains AM4001, AM4011, AM4012, AM4017, AM4023, and AM4028 have an LPS corresponding to the LPS from the reference strain D21e7, which is galactose and phosphate deficient (3; Fig. 3). This observation is in agreement with the observed C21 sensitivity of these mutants (Table 2) as well as with the observation that the amount of protein b in cells grown in peptone-yeast broth is strongly decreased in mutants lacking heptose-bound phosphate (16b). Figure 3 shows that strains AM4014 and AM4018 contain mutant LPS such that the R_f value is lower than the R_f value of galactoseless LPS of reference strain CE1007. This suggests that galactose is present, which is in agreement with the

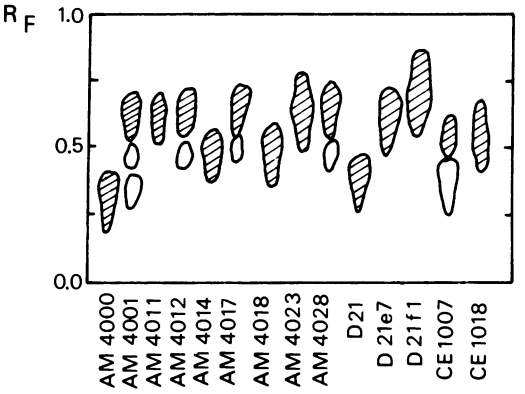

FIG. 3. *Schematic representation of chromatographic behavior of LPS of the parental strain AM4000, the mutant strains, and some LPS mutants. Shaded and white spots indicate major and minor components, respectively. The R_f value of inorganic phosphate is about 0.35.*

resistance of these mutants to phage C21. The mutant strains AM4001, AM4012, AM4017, and AM4028 also have a small amount of LPS corresponding to the LPS of strains AM4014 and AM4018.

Recipient ability of some LPS mutants in crosses with I- and F-type donors. The mutants described in this paper, all isolated in a direct way, appear to be LPS mutants. We examined the recipient ability of some reference LPS mutants in crosses with I- and F-type donors. Table 4 shows that strains CE1007 and CE1018, with galactose- and glucose-deficient LPS, respectively, are good recipients in matings with both types of donor strains. Strain D21e7, with galactoseless and heptose-bound, phosphateless LPS, is a poor recipient with an I-type donor but has a wild-type recipient ability with an F-type donor. Glucose and phosphateless LPS (strain D21f1) results in poor

TABLE 4. *Recipient ability of some LPS mutants in crosses with an I-type and an F-type donor*[a]

Recipient	LPS character	Donor[b]	
		I-type	F-type
CE1007	Galactoseless	+	+
CE1018	Glucoseless	+	+
D21e7	Galactoseless and deficient in heptose-bound phosphate	−	+
D21f1	Glucoseless and deficient in heptose-bound phosphate	−	−
Several[c]	Heptoseless	+ or −	−

[a] + and − stand for Con$^+$ and Con$^-$, respectively.

[b] Strain I53 was used as the I-type donor strain; strain PC0031 was used as the F-type donor strain.

[c] Strains PC2040, PC2041 (9), and D21f2 (4) are Con$^+$ in crosses with strain I53 as the donor strain; strains CE1032, CE1035, CE1037, CE1040, and CE1042 (22a) are Con$^-$ in crosses with I53 as the donor strain.

recipient ability with both types of donor strains. Surprisingly, it appears from Table 4 that some mutants with heptoseless LPS are good recipients with an I-type donor, whereas others are conjugation deficient. All heptose-deficient mutants tested so far are Con$^-$ with F-type donors.

Effect of LPS on the conjugation of recipient cells with an I-type donor. All ConI$^-$ mutants described in this paper are LPS mutants, and therefore it would be interesting to study whether isolated LPS can inhibit matings with an I-type donor strain. Figure 4 shows that addition of wild-type LPS (1 mg/ml, final concentration) to a mating mixture with an I-type donor reduces the transconjugant formation frequency about 150 times, whereas the addition of LPS isolated from mutant strain AM4014 results only in about a threefold reduction. The addition of wild-type or mutant LPS did not significantly inhibit matings with an F-donor strain (HfrR4, results not shown).

Tentative location of the *conI* mutations. When about 10^8 cells of strain AM4001, AM4011, AM4012, AM4017, AM4023, or AM4028 were plated on peptone-yeast agar containing 1% (wt/vol) SDS, a number of surviving colonies were found. These colonies were revertants in that they were ConI$^+$ and identical to the parental strain AM4000 in phage pattern. To locate the *conI* mutation, we looked for the chromosomal site of sensitivity to SDS. Transconjugants from crosses with several Hfr strains and the appropriate ConI$^-$ recipients were analyzed for sensitivity to SDS. Since strains AM4014 and AM4018 are resistant to 1% (wt/vol) SDS, transconjugants of these strains were examined directly for their ConI character.

Genetic analysis of *argE*$^+$ *strA* transconjugants from a cross of the donor strain Hfr KL14 and the mutant strains AM4001, AM4011, AM4012, AM4017, and AM4028, respectively, showed that sensitivity to SDS in these mutants is linked more closely to *argE* (about 50%) than *xyl* is linked to *argE* (about 30%). Analysis of *xyl*$^+$ Tet^r transconjugants of the tetracycline-resistant derivatives of the mutants with Hfr AB313 showed that the SDS sensitivity in the mutant strains AM4001, AM4011, AM4012, AM4017, and AM4028 is closely linked to *xyl* (about 80%). We therefore suggest that the *conI* mutations in these strains map in the 78- to 82-min region of the *E. coli* map (2). Analysis of *xyl*$^+$ Tet^r transconjugants of a tetracycline-resistant derivative of the mutant strain AM4014 with the donor strain Hfr AB313 showed that about 60% of these transconjugants were ConI$^+$. Crosses of strain AM4018 with the donor strains Hfr KL14 and Hfr AB313 suggested also that in strain AM4018 the *conI* mutation maps in the 80-min region. Analysis of transconju-

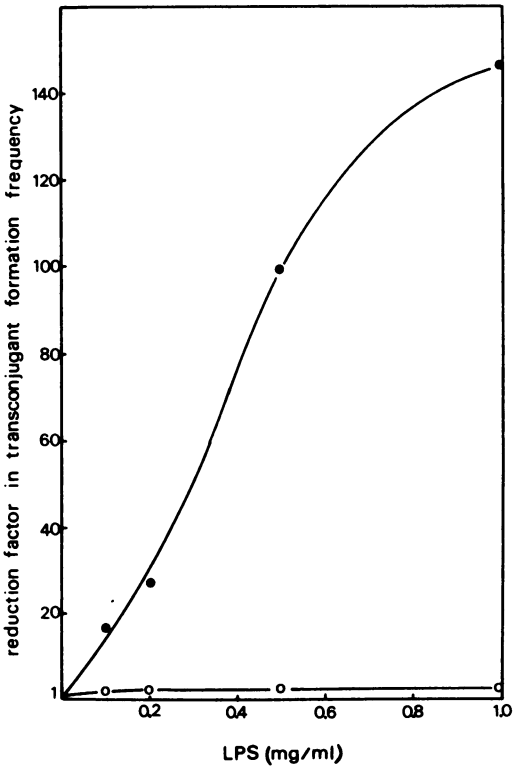

FIG. 4. *Inhibition of transconjugant formation frequency by LPS isolated from the parental strain AM4000 (●) and from the ConI$^-$ mutant strain AM4014 (○). Strains I53 and AM4000 were used as donor and recipient strains, respectively.*

gants from crosses of strain AM4023 with several Hfr strains, such as HfrH, Hfr Cav, and Hfr KL16, suggested that the mutation in strain AM4023 maps near *gal*. Furthermore, 98 out of 98 *gal*$^+$ transductants, from a transduction with P1 propagated on a *gal*$^+$, ConI$^+$ donor and strain AM4023 as a recipient, appeared to be SDS resistant. The *conI* mutation in strain AM4023 apparently maps very near or in the *gal* operon.

DISCUSSION

Until now only Con$^-$ mutants deficient in crosses with F-type donor strains (ConF$^-$) have been described. Most of them were isolated indirectly after preselection for phage resistance (9, 20, 22) or antibiotic resistance (17). Only one was isolated in a specific enrichment procedure (8). Surprisingly, all ConF$^-$ mutants tested so far, except mutant FR3A (20), are ConI$^+$, indicating that the receptors used for conjugation by F- and I-type donors are different. Therefore, we attempted the isolation of specific ConI$^-$ mutants. All ConI$^-$ mutants we have isolated are indeed specifically impaired in crosses with a donor carrying I-pili (Table 3).

Our results strongly suggest that the mutants are impaired in the formation of preliminary or effective mating pairs. This idea seems to be reasonable, since: (i) the ConI$^-$ mutants are specifically impaired in conjugation with I-type donors (Table 3), and it is likely that steps in the part of the conjugation process after mating pair formation are independent of the type of donor; (ii) the ability to form mating pairs was tested for some mutants according to the technique described by Skurray et al. (22) (pair formation was not detected; results not shown); and (iii) the mutants contain an altered LPS, a component exclusively located at the cell surface (18).

After growth in brain heart broth, all ConI$^-$ mutants lack the outer-membrane protein b. Some ConI$^-$ mutants have a wild-type recipient ability when cells are cultivated in peptone-yeast broth, whereas protein b is still absent. Therefore, we conclude that protein b is not involved in recipient ability in crosses with an I-type donor. Its absence probably is the consequence of a pleiotropic effect, as was observed earlier for some LPS mutants (16b). The fact that Henning's mutant strain P6922dI, lacking proteins b, c, and d (10) after growth in brain heart broth, is a good recipient in crosses with an I-type donor (results not shown) strongly supports this statement.

All the ConI$^-$ mutants are LPS mutants. The chromatographic mobilities of LPS of the mutants suggest that strains AM4014 and AM4018 are impaired in later steps of LPS biosynthesis than are the other mutants. If LPS is directly involved in recipient ability in crosses with an I-type donor, one expects that all mutations blocking the biosynthesis of the LPS earlier than the mutations in AM4014 and AM4018 are ConI$^-$ mutants. Our results (see Table 4), however, showed that there is no simple correlation between the site of the mutation in the LPS biosynthesis and the ConI character.

There are three possibilities to explain the involvement of LPS of the recipient cell in the mating ability with an I-type donor: (i) LPS is the receptor for the I-pilus; (ii) LPS is not the receptor for the I-pilus, but some steps in the LPS biosynthesis are also involved in the biosynthesis of the receptor; and (iii) some changes in the LPS structure may lead to a conformational change of the receptor site, leading to a loss of receptor activity.

The fact that LPS inhibits the conjugation with an I-type donor strongly suggests that LPS is the receptor for the I-pilus. Lancaster et al. (13) and Cartwright (Ph.D. thesis, Univ. of Oklahoma, Norman, 1971) found that LPS inhibits the conjugation with an F-type donor two to three times using broth as the growth medium. They suggested, therefore, that LPS is the conjugation factor for crosses with an F-type donor. We also found a two- to threefold reduction in transconjugant formation by adding LPS in the case of an F-type donor. Our results from crosses with an I-type donor, which gave a 150-fold reduction in transconjugant formation after addition of LPS, predict a similar high reduction in F-crosses, provided that LPS is an F conjugation factor. The finding that the effect is small suggests that it is trivial.

Characterization of mutant LPS by comparison of its chromatographic mobility with that of reference LPS might be misleading, as the LPS's of the two parent strains differ in R_f value. Chemical analysis of LPS will be required to establish the LPS structure of the mutants. However, the chromatographic behavior of LPS gives the following useful information: (i) it indicates which LPS's are nonidentical; and (ii) it shows that mutants might be leaky. The *galE* strain CE1007 gives two types of LPS (Fig. 3). Analysis of its sugar composition indicates that it contains some galactose. Strain AM4001 also contains a component corresponding to wild-type LPS (Fig. 3), suggesting that it is leaky. Four strains, AM4001, AM4012, AM4017, and AM4028, contain a component with a chromatographic mobility between that of wild-type LPS and the majority of its mutant LPS. This unknown component is nonidentical with inorganic phosphate since this has a lower

R_f value. Experiments in which various kinds of mutant LPS's, isolated from cells grown in various media, are used as conjugation inhibitors, are in progress. Chemical analysis of the LPS of the mutants is required to give insight into the exact requirements for the I-type donor receptor.

The mutants described in this paper are phenotypically different. Their mutations all map near *xyl* except the mutation of strain AM4023, which maps very near *gal* and might in fact be a *gal* mutation. Strains PC2041 (9) and FR19B (20) are heptose-deficient mutants and also map near *xyl*. These mutants, however, are ConF$^-$. Furthermore, the *rfa* gene is also located near *xyl*. These facts taken together suggest that there might be a cluster of LPS genes in the 80-min region on the *E. coli* K-12 chromosomal map (2).

From the preliminary genetical characterization and biochemical analysis of the mutants described in this paper, it is clear that ConI$^-$ mutants differ from ConF$^-$ mutants. Previous results (8, 9, 11, 22) suggest that one of the major outer-membrane proteins, protein d, is the receptor for the F-pilus; the results presented in this paper strongly suggest that LPS is the receptor for the I-pilus.

LITERATURE CITED

1. **Ames, G. F., E. N. Spudich, and H. Nikaido.** 1974. Protein composition of the outer membrane of *Salmonella typhimurium*: effect of lipopolysaccharide mutations. J. Bacteriol. 117:406–416.
2. **Bachmann, B. J., K. B. Low, and A. L. Taylor.** 1976. Recalibrated linkage map of *Escherichia coli* K-12. Bacteriol. Rev. 40:116–167.
3. **Boman, H. G., and D. A. Monner.** 1975. Characterization of lipopolysaccharide from *Escherichia coli* K-12 mutants. J. Bacteriol. 121:455–464.
4. **Braun, V., and H. Wolff.** 1973. Characterization of the receptor protein for phage T5 and colicin M in the outer membrane of *E. coli* B. FEBS Lett. 34:77–80.
5. **Curtiss, R., L. J. Charamella, D. R. Stallion, and J. A. Mays.** 1968. Parental functions during conjugation in *Escherichia coli* K-12. Bacteriol. Rev. 32:320–348.
6. **Foulds, J.** 1974. Chromosomal location of the *tolG* locus for tolerance to bacteriocin JF246 in *Escherichia coli* K-12. J. Bacteriol. 117:1354–1355.
7. **Galanos, C., O. Lüderitz, and O. Westphal.** 1969. A new method for the extraction of R lipopolysaccharides. Eur. J. Biochem. 9:245–249.
8. **Havekes, L. M., and W. P. M. Hoekstra.** 1976. Characterization of an *Escherichia coli* K-12 F$^-$Con$^-$ mutant. J. Bacteriol. 126:593–600.
9. **Havekes, L. M., B. J. J. Lugtenberg, and W. P. M. Hoekstra.** 1976. Conjugation deficient *E. coli K12* F$^-$ mutants with heptose-less lipopolysaccharide. Mol. Gen. Genet. 146:43–50.
10. **Henning, U., and I. Haller.** 1975. Mutants of *Escherichia coli* K12 lacking all "major" proteins of the outer cell envelope membrane. FEBS Lett. 55:161–164.
11. **Henning, U., I. Hindennach, and I. Haller.** 1976. The major proteins of the *Escherichia coli* outer cell envelope membrane: evidence for the structural gene of protein II*. FEBS Lett. 61:46–48.
12. **Koplow, J., and H. Goldfine.** 1974. Alterations in the outer membrane of the cell envelope of heptose-deficient mutants of *Escherichia coli*. J. Bacteriol. 117:527–543.
13. **Lancaster, J. H., E. P. Goldschmidt, and O. Wyss.** 1965. Characterization of conjugation factors in *Escherichia coli* cell walls. I. Inhibition of recombination by cell walls and cell extracts. J. Bacteriol. 89:1478–1481.
14. **Lindberg, A. A.** 1973. Bacteriophage receptors. Annu. Rev. Microbiol. 27:205–241.
15. **Low, K. B.** 1972. *Escherichia coli* K-12 F-prime factors, old and new. Bacteriol. Rev. 36:587–607.
16. **Low, K. B., and T. H. Wood.** 1965. A quick and efficient method for interruption of bacterial conjugation. Genet. Res. 6:300–303.

16a. **Lugtenberg, B., J. Meijers, R. Peters, P. van den Hoek, and L. van Alphen.** 1975. Electrophoretic resolution of the "major outer membrane protein" of *Escherichia coli* K-12 into four bands. FEBS Lett. 58:254–258.

16b. **Lugtenberg, B., R. Peters, H. Bernheimer, and W. Berendsen.** 1976. Influence of culture conditions and mutations on the composition of the outer membrane proteins of *Escherichia coli*. Mol. Gen. Genet. 147: 251–262.

17. **Monner, D. A., S. Jonsson, and H. G. Boman.** 1971. Ampicillin-resistant mutants of *Escherichia coli* K-12 with lipopolysaccharide alterations affecting mating ability and susceptibility to sex-specific bacteriophages. J. Bacteriol. 107:420–432.
18. **Mühlradt, P. F., and J. R. Golecki.** 1975. Asymmetrical distribution and artifactual reorientation of lipopolysaccharide in the outer membrane bilayer of *Salmonella typhimurium*. Eur. J. Biochem. 51:343–352.
19. **Prehm, P., S. Stirm, B. Jann, and K. Jann.** 1975. Cell wall lipopolysaccharide from *Escherichia coli* B. Eur. J. Biochem. 56:41–55.
20. **Reiner, A. M.** 1974. *Escherichia coli* females defective in conjugation and in adsorption of a single-stranded deoxyribonucleic acid phage. J. Bacteriol. 119:183–191.
21. **Schade, S. Z., J. Adler, and H. Ris.** 1967. How bacteriophage χ attacks motile bacteria. J. Virol. 1:599–609.
22. **Skurray, R. A., R. E. W. Hancock, and P. Reeves.** 1974. Con$^-$ mutants: class of mutants in *Escherichia coli* K-12 lacking a major cell wall protein and defective in conjugation and adsorption of bacteriophage. J. Bacteriol. 119:726–735.

22a. **van Alphen, W., B. Lugtenberg, and W. Berendsen.** 1976. Heptose-deficient mutants of *Escherichia coli* K12 deficient in up to three major outer membrane proteins. Mol. Gen. Genet. 147:263–269.

23. **Verkley, A. J., E. J. J. Lugtenberg, and P. H. J. Th. Ververgaert.** 1976. Freeze etch morphology of outer membrane mutants of *Escherichia coli* K12. Biochim. Biophys. Acta 426:581–586.
24. **Wilkinson, R. G., P. Gemski, and B. A. D. Stocker.** 1972. Non smooth mutants of *Salmonella typhimurium*: differentiation by phage sensitivity and genetic mapping. J. Gen. Microbiol. 70:527–554.
25. **Willetts, N. S., A. J. Clark, and K. B. Low.** 1969. Genetic location of certain mutations conferring recombination deficiency in *Escherichia coli*. J. Bacteriol. 97:244–249.
26. **Winkler, K. C., and P. G. de Haan.** 1948. On the action of sulfanilamide. XII. A set of noncompetitive sulfanilamide antagonists for *Escherichia coli*. Arch. Biochem. 18:97–107.